the storage, distribution and illustration of morphological characters of nematodes. Journal of Nematology **34:** 296–302.
DURAN, E., D. J. WALKER, K. R. JOHNSON, P. R. KOMUNIECKI, AND R. W. KOMUNIECKI. 1998. Developmental and tissue-specific expression of 2-methyl branched-chain enoyl CoA reductase isoforms in the parasitic nematode, *Ascaris suum*. Molecular and Biochemical Parasitology **91:** 307–18.
HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics **17:** 754–755.
KATINKA, M. D., S. DUPRAT, E. CORNILLOT, G. METENIER, F. THOMARAT, G. PRENSIER, V. BARBE, E. PEYRETAILLADE, P. BROTTIER, P. WINCKER, F. DELBAC, H. EL ALAOUI, P. PEYRET, W. SAURIN, M. GOUY, J. WEISSENBACH, AND C. P. VIVARES. 2001. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. Nature **414:** 450–453.
POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics **14:** 817–818.
SIDDALL, M. E., D. R. BROOKS, AND S. S. DESSER. 1993. Phylogeny and the reversibility of parasitism. Evolution **47:** 308–313.
SWOFFORD, D. L. 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, Massachusetts.
TANDINGAN DE LEY, I., P. DE LEY, A. VIERSTRAETE, G. KARSSEN, M. MOENS, AND J. VANFLETEREN. 2002. Phylogenetic analysis of *Meloidogyne* SSU rDNA. Journal of Nematology **34:** 319–327.
THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research **25:** 4876–4882.
WIJOVÁ, M., F. MORAVEC, A. HORÁK, D. MODRÝ, AND J. LUKEŠ. 2005. Phylogenetic position of *Dracunculus medinensis* and some related nematodes inferred from 18S rRNA. Parasitology Research **96:** 133–135.

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# Seroprevalence of *Toxoplasma gondii* Antibodies in Humans From Rural Western Amazon, Brazil

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ABSTRACT: Antibodies to *Toxoplasma gondii* were assayed in sera of 266 humans from 71 farms located at Rondônia State, Western Amazon, Brazil, by the modified agglutination test (MAT) and the indirect immunofluorescent antibody test (IFAT). Antibodies were found in 195 humans (73.3%), with MAT titers of 1:25 in 11, 1:50 in 11, 1:100 in 16, 1:200 in 27, 1:400 in 38, 1:800 in 37, 1:1,600 in 22, and 1:3,200 or higher in 33. From the 71 farms visited, 69 had seropositive humans. Prevalence of anti-*T. gondii* antibodies increased with age of the people ($P < 0.05$), and no difference was observed in the occurrence by gender ($P > 0.05$). A sanitary questionnaire was applied in each farm, and statistical association between the serologic status and several variables were analyzed. Home-grown vegetable consumption and origin of drinking water (well or river) were the independent variables that displayed significant association ($P = 0.002$ and 0.02, respectively). Higher values of occurrence were found in people with consumption of home-grown vegetables (76.1%) and people that drink well water (75.4%) compared with people that did not consume this type of food (61.9%) and drink river water (55.2%). By IFAT (≥1:16), 194 of 266 (73%) humans were seropositive and there was a good correlation between MAT and IFAT.

*Toxoplasma gondii* infection is widely prevalent in human beings and other animals worldwide (Dubey and Beattie, 1988; Tenter et al., 2000; Remington et al., 2001). Humans become infected postnatally by ingesting tissue cysts from undercooked meat, consuming food or drink contaminated with oocysts, or by accidentally ingesting oocysts from the environment. However, only a small percentage of exposed adult humans develop clinical signs. It is unknown whether the severity of toxoplasmosis in immunocompetent persons is because of the parasite strain, host variability, or other factors. However, in Erechim, southern Brazil, 17.7% of 1,042 adults were found to have retinal scars, presumably caused by *T. gondii* (Glasner et al., 1992). Serologic surveys indicate that *T. gondii* infection is endemic in Brazil (Sobral et al., 2005, and references therein). Recent studies indicate that the strains of *T. gondii* prevalent in chickens and cats from Brazil are biologically and genetically different from the samples from North America (Dubey et al., 2002, 2003, 2006; Pena et al., 2006).

In the present study, we have surveyed 266 humans from Amazon, Brazil, for *T. gondii* antibodies. This location is geographically, socially, and ecologically different from other parts of the world.

Humans surveyed were from Monte Negro County, Rondônia State, western Amazon, Brazil (10°15′35″S, 63°18′06″W). It has a population of approximately 13,000, most of them living in the rural area on small, family-employed farms with very close contact among humans and animals. The region has a hot and humid climate, with high levels of precipitation that average 2,000 mm annually, with a moderate drought period from April to October. Temperature ranges from 25 to 29 C with a relative humidity of 70–80% throughout the year (Camargo et al., 2002).

From 722 farms registered in the area surveyed, 86 were randomly selected (confidence level = 95%, precision = 10%), and the people living in those farms were invited to collaborate in this study (Thrusfield, 1995). A signed consent form was obtained from all adults as well from the parents or legal guardians of minors who participated in this survey. From the 86 randomly selected farms, on 71 properties, permission was obtained for blood sample collection.

Serum samples were collected from 266, 8-mo to 76-yr-old humans from May to October 2002 and stored at −20 C until serologic analysis. During the visit to each farm, a questionnaire was applied on the farm owner or supervisor, always by the same person for standardization. The independent variables studied were presence of cat, meat consumption, hunting meat consumption, home-grown vegetables, drinking water origin, water storage, presence of filtered water, and water treatment.

Serum samples were initially screened for *T. gondii* antibodies by using a 1:16 dilution of serum in an indirect immunofluorescence antibody test (IFAT; Camargo, 1974) and then tested further by 2-fold dilutions (1:25–1: 3,200) by the modified agglutination test (MAT; Dub-

TABLE I. Antibody titers to *Toxoplasma gondii* in sera of 266 humans from Monte Negro County, Rondônia State, Brazilian Western Amazon, by the modified agglutination test (MAT).

| Age (yr) | No. tested | No. positive (%) | MAT titer | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 25 | 50 | 100 | 200 | 400 | 800 | 1,600 | ≥3,200 |
| 0–6 | 19 | 7 (3.6) | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 4 |
| 7–12 | 29 | 18 (9.2) | 0 | 1 | 0 | 1 | 4 | 1 | 5 | 6 |
| 13–19 | 49 | 30 (15.4) | 0 | 1 | 1 | 2 | 4 | 6 | 4 | 12 |
| 20–40 | 74 | 64 (32.8) | 5 | 2 | 6 | 13 | 13 | 17 | 4 | 4 |
| >40 | 95 | 76 (39.0) | 6 | 7 | 9 | 11 | 16 | 12 | 8 | 7 |
| Total | 266 | 195 | 11 | 11 | 16 | 27 | 38 | 37 | 22 | 33 |

ey and Desmonts, 1987). Sera with discrepant findings by MAT and IFAT were tested by the dye test (DT) as described by Desmonts and Remington (1980). Epidiomologic evaluation was based on the MAT.

The independent variables were subjected to univariate analysis by chi-square test ($\chi^2$) or Fisher exact test (Hosmer and Lemeshow, 1989). It was not possible to run a multiple analysis because 69 of 71 farms had cases of toxoplasmosis. Thus, the univariate analysis was performed with the results from each sampled person. A Kappa test was used to compare the IFAT and MAT assays. Analyses were performed using SPSS for Windows. For evaluation of the differences between age groups, occurrence values of the $\chi^2$ for trend was used with EpiInfo version 6.0 for Windows.

*Toxoplasma gondii* antibodies were found in 195 (73.3%) of 266 sera, with sera with MAT titers of 1:25 in 11, 1:50 in 11, 1:100 in 16, 1:200 in 27, 1:400 in 38, 1:800 in 37, 1:1,600 in 22, and 1:3,200 or higher in 33 sera (Table I). IFAT antibodies were found 195 of 266 (73.3%) sera. There was 100% agreement between the MAT and DT and IFAT in general; 2 sera negative by IFAT were positive by DT and MAT, and 1 serum positive by IFAT was negative by DT and MAT. From the 71 farms where blood samples were collected, 68 (95.8%) of the farms had at least 1 person with positive result by MAT. The samples were stratified by gender and age to evaluate the occurrence of *T. gondii* seroantibodies. In MAT, the occurrence in males and females was 68.8% (97/141) and 78.4% (98/125), respectively. No statistical differences were observed between genders ($P > 0.05$); however, the number of infected people increased with age ($\chi^2 = 11.8$; $P < 0.0$; $r^2 = 0.047$).

TABLE II. Statistical analyses of association between studied variables and presence of antibody anti-*Toxoplasma gondii* in humans from rural population from Western Amazon, Brazil.

| | No. of humans | | | Analyses* | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Univariate | | Multivariate | | |
| Variable | Examined | Positive | % | $\chi^2$ | *P* | OR | *P* | 95% CI |
| Presence of cat | | | | | | | | |
| No | 122 | 89 | 72.9 | | | | | |
| Yes | 144 | 106 | 73.6 | 0.015 | 0.9 | | | |
| Meat consumption | | | | | | | | |
| No | 12 | 7 | 58.3 | | | | | |
| Yes | 247 | 181 | 73.3 | 1.28 | 0.26 | | | |
| Hunting | | | | | | | | |
| No | 100 | 79 | 79.0 | | | | | |
| Yes | 159 | 109 | 68.5 | 3.37 | 0.07 | | | |
| Home-grown vegetables | | | | | | | | |
| No | 92 | 57 | 61.9 | | | | | |
| Yes | 172 | 136 | 76.1 | 8.93 | 0.003 | 2.48 | 0.002 | (1.39, 4.42) |
| Drinking water | | | | | | | | |
| Well | 228 | 172 | 75.4 | | | | | |
| River | 29 | 16 | 55.2 | 5.38 | 0.02 | 0.38 | 0.02 | (0.17, 0.85) |
| Water filter | | | | | | | | |
| No | 28 | 23 | 82.1 | | | | | |
| Yes | 229 | 165 | 72.0 | 1.29 | 0.25 | | | |
| Water storage | | | | | | | | |
| Rainfall | 124 | 89 | 71.8 | | | | | |
| Buckets | 133 | 99 | 74.4 | 0.23 | 0.63 | | | |
| Water treatment | | | | | | | | |
| No | 124 | 89 | 71.8 | | | | | |
| Yes | 133 | 99 | 74.4 | 0.23 | 0.63 | | | |

* CI, confidence interval; OR, odd ratio.

The results of univariate analyses are given in Table II. Hunting, home-grown vegetables, and source of drinking water were the independent variables that displayed significant association ($P < 0.2$) by the univariate analysis. However, using the multivariate analyses, only home-grown vegetables and drinking water origin presented association with the presence of *T. gondii* antibodies ($P < 0.05$).

Seroprevalences of *T. gondii* are very high in many regions of Brazil, including areas where Indian tribes live in relative isolation from civilization. Lovelace et al. (1978) conducted a survey of the Ticuna and Codaja Amazon Indian tribes, and prevalences of 77 and 39% were obtained, respectively. Ferraroni et al. (1982) studied 5 populations in the Brazilian Amazon and obtained prevalences that ranged from 56 to 74%. Three of these populations were also from rural area (56, 66, and 70%). In a recent study of Indians tribes from Pará and Amapá states, also in the Amazon region, the seroprevalences were 55.6% and 59.6%, respectively. Our data from the immigrant population in Amazon revealed a similar prevalence.

The increasing seroprevalence of *T. gondii* with age of the examined persons ($P < 0.05$), indicates a postnatal acquisition of infection and supports the findings of Kobayashi et al. (2002) in Pernambuco, and by Garcia et al. (1999) in Paraná, respectively, in the northeastern and southern regions of Brazil. No difference was observed between the occurrence values by sex or in populations from rural areas by Kobayashi et al. (2002) and Garcia et al. (1999).

Eating home-grown vegetables and drinking well water were the variables that presented association with *T. gondii* infection. In this survey, *T. gondii* prevalence data had no association with the presence of cats; however, the presence of wild felids is common on farms of the region, and they could represent a source of oocyst contamination. Silva et al. (2002) described the presence of antibody anti-*T. gondii* in a variety of Neotropical felid species from Brazil.

In a high endemic area, and especially with a poor hygienic environment, it is difficult to accurately access the risk factors. Nevertheless, the consumption of home-grown vegetables and drinking well water had a significant association with prevalence of *T. gondii* antibodies. The isolation of viable *T. gondii* from 24 of 50 free-range chickens from the same area as surveyed in the present study (Dubey et al., 2006) indicates a heavy contamination of the environment with oocysts. These observations, associated with the presence of antibodies anti-*T. gondii* in humans from Indian tribes that do not eat red meat (Sobral et al., 2005), support the observation of Bahia-Oliveira et al. (2003) and de Moura et al. (2006) that ingestion of *T. gondii* oocysts is the main mode of transmission of *T. gondii* in Brazil.

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## LITERATURE CITED

BAHIA-OLIVEIRA, L. M. G., J. L. JONES, J. AZEVEDO-SILVA, C. C. F. ALVES, F. ORÉFICE, AND D. G. ADDISS. 2003. Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro State, Brazil. Emerging Infectious Diseases **9:** 55–62.

CAMARGO, L. M. A., M. M. MOURA, V. ENGRACIA, R. C. PAGOTTO, S. A. BASANO, L. H. PEREIRA DA SILVA, E. P. CAMARGO, B. BEIGUELMAN, AND H. KRIEGER. 2002. A rural community in a Brazilian Western Amazonian region: Some demographic and epidemiological patterns. Memórias do Instituto Oswaldo Cruz **97:** 193–195.

CAMARGO, M. E. 1974. Introdução às técnicas de imunofluorescência. Revista Brasileira de Patologia Clínica **10:** 143–171.

DE MOURA, L., L. M. G. BAHIA-OLIVEIRA, M. Y. WADA, J. L. JONES, S. H. TUBOI, E. H. CARMO, W. M. RAMALHO, N. J. CAMARGO, R. TREVISAN, R. M. T. GRAÇA, A. J. DA SILVA, I. MOURA, J. P. DUBEY, AND D. O. GARRETT. 2006. Waterborne toxoplasmosis, Brazil, from field to gene. Emerging Infectious Diseases **12:** 326–329.

DUBEY, J. P., AND C. P. BEATTIE. 1988. Toxoplasmosis of animals and man. CRC Press, Boca Raton, Florida, 220 p.

———, AND G. DESMONTS. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Veterinary Journal **19:** 337–339.

———, S. M. GENNARI, M. B. LABRUNA, L. M. A. CAMARGO, M. C. B. VIANNA, P. L. MARCET, AND T. LEHMANN. 2006. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Amazon, Brazil. Journal of Parasitology **92:** 36–40.

———, D. H. GRAHAM, C. R. BLACKSTON, T. LEHMANN, S. M. GENNARI, A. M. A. RAGOZO, S. M. NISHI, S. K. SHEN, O. C. H. KWOK, D. E. HILL, AND P. THULLIEZ. 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: Unexpected findings. International Journal for Parasitology **32:** 99–105.

———, ———, D. S. DA SILVA, T. LEHMANN, AND L. M. G. BAHIA-OLIVEIRA. 2003. *Toxoplasma gondii* isolates of free-ranging chickens from Rio de Janeiro, Brazil: Mouse mortality, genotype, and oocyst shedding by cats. Journal of Parasitology **89:** 851–853.

FERRARONI, J. J., S. G. REED, AND C. A. SPEER. 1982. Prevalence of *Toxoplasma* antibodies in humans and various animals in the Amazon. Proceedings of the Helminthological Society of Washington **47:** 148–150.

GARCIA, J. L., I. T. NAVARRO, L. OGAWA, AND R. C. OLIVEIRA. 1999. Soroprevalência do *T. gondii* em suinos, bovinos, ovinos e equinos, e sua correlação com humanos, felinos e caninos, oriundos de propriedades rurais do norte do Paraná-Brasil. Ciência Rural Santa Maria, **29:** 91–97.

GLASNER, P. D., C. SILVEIRA, D. KRUSZON-MORAN, M. C. MARTINS, M. BURNIER, S. SILVEIRA, M. E. CAMARGO, R. B. NUSSENBLATT, R. A. KASLOW, AND R. BELFORT. 1992. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. American Journal of Ophthalmology **114:** 136–144.

HOSMER, JR., D. W., AND S. LEMESHOW. 1989. Applied logistic regression, 2nd edition. Wiley, New York, 307 p.

KOBAYASHI, M., E. MALAGUENO, J. V. SANTANA, E. PEREZ, AND A. YANO. 2002. Prevalence of toxoplasmosis in children northeastern Brazil. Japanese Journal of Tropical Medicine and Hygiene **30:** 305–310.

LOVELACE, J. K., M. A. P. MORAES, AND E. HAGERBY. 1978. Toxoplasmosis among the Ticuna Indians in the State of Amazonas, Brazil. Tropical and Geographical Medicine **30:** 295–300.

PENA, H. F. J., R. M. SOARES, M. AMAKU, J. P. DUBEY, AND S. M. GENNARI. 2006. *Toxoplasma gondii* infection in cats from São Paulo State, Brazil: Seroprevalence, oocyst shedding, isolation in mice, and biologic and molecular characterization. Research in Veterinary Science **81:** 58–67.

REMINGTON, J. S., R. MCLEOD, P. THULLIEZ, AND G. DESMONTS. 2001. Toxoplasmosis. *In* Infectious diseases of the fetus and newborn, 5th ed., J. S. Remington and J. Klein (eds.). Saunders, Philadelphia, p. 205–346.

SILVA, J. C. R., S. OGASSAWARA, C. H. ADANIA, F. FERREIRA, S. M. GENNARI, J. P. DUBEY, AND J. S. FERREIRA-NETO. 2002. Seroprevalence of *Toxoplasma gondii* in captive Neotropical felids from Brazil. Veterinary Parasitology **102:** 217–224.

SOBRAL, C. A., M. R. R. AMENDOEIRA, A. TEVA, B. N. PATEL, AND C. H. KLEIN. 2005. Seroprevalence of infection with *Toxoplasma gondii* in indigenous Brazilian populations. American Journal of Tropical Medicine and Hygiene **72:** 37–41.

TENTER, A. M., A. R. HECKEROTH, AND L. M. WEISS. 2000. *Toxoplasma gondii*: From animals to humans. International Journal for Parasitology **30:** 1217–1258.

THRUSFIELD, M. 1995. Veterinary epidemiology, 2nd ed. Blackwell Scientific, Oxford, U.K., 479 p.